

**FIG. 1A****Nucleotide sequence of EOS (SEQ.ID.NO1)**

CCACGCGTCCGACCAGAGTCCAAGCCCTAGGCAGTGCCACCCTTACCCAGCCCAGCCTTG  
AAGACAGAATGAGAGGGGTTTCCTGTCTCCAGGTCTGCTCCTTCTGGTGCTGGGAGCTG  
CTGGGACTCAGGGAAGGAAGTCTGCAGCCTGCGGGCAGCCCCGCATGTCCAGTCGGATCG  
TTGGGGGCCGGGATGGCCGGGACGGAGAGTGGCCGTGGCAGGCGAGCATCCAGCATCCTG  
GGGCACACGTGTGCGGGGGGTGCTCATGCCCCCAGTGGGTGCTGACAGCGGCGCACT  
GCTTCCCCAGGAGGGCACTGCCAGCTGAGTACCGCGTGCGCCTGGGGGCGCTGCGTCTGG  
GCTCCACCTCGCCCCGCACGCTCTCGGTGCCCCGTGCGACGGGTGCTGCTGCCCCCGGACT  
ACTCCGAGGACGGGGCCCCGCGGCGACCTGGCACTGCTGCAGCTGCGTCCGCCGGTGCCCC  
TGAGCGCTCGCGTCCAACCCGTCTGCCTGCCCCGTGCCCCGGCGCCCCGCCGCCCGGCA  
CACCATGCCGGGTACCCGGCTGGGGCAGCCTCCGCCCAGGAGTGCCCCCTCCCAGAGTGGC  
GACCGCTACAAGGAGTAAGGGTGCCGCTGCTGGACTCGCGCACCTGCGACGGCCTCTACC  
ACGTGGGCGCGGACGTGCCCCAGGCTGAGCGCATTGTGCTGCCTGGGAGTCTGTGTGCCG  
GCTACCCCCAGGGCCACAAGGACGCTGCCAGGGTGATTCTGGGGGACCTCTGACCTGCC  
TGCAGTCTGGGAGCTGGGTCTGGTGGGCGTGGTGAAGTGGGGCAAGGGTTGTGCCCTGC  
CCAACCGTCCAGGGGTCTACACCAGTGTGGCCACATATAGCCCCCTGGATTTCAGGCTCGCG  
TCACTTCTAATGCTAGCCGGTGAGGCTGACCTGGAGCCAGCTGCTGGGGTCCCTCAGCCT  
CCTGGTTTCATCCAGGCACCTGCCTATACCCACATCCCTTCTGCCTCGAGGCCAAGATGC  
CTAAAAAAGCTAAAGGCCACCCACCCCCACCCACCACCTTCTGGCTCCTCTCCTCTTT  
GGGGATCACCAGCTCTGACTCCACCAACCCTCATCCAGGAATCTGCCATGAGTCCCAGGG  
AGTCACACTCCCCACTCCCTTCCTGGCTTGTATTACTTTTCTTGGCCCTGGCCAGGGCT  
GGGCGCAAGGCACGCAGTGATGGGCAAACCAATTGCTGCCCATCTGGCCTGTGTGCCCAT  
CTTTTTCTGGAGAAAGTCAGATTTCACAGCATGACAGAGATTTGACACCAGGGAGATCCTC  
CATAGCTGGCTTTGAGGACACGGGGACCACAGCCATGAGCGGCCTCTAAGAGCTGAGAGA  
CAGCCGGCAGGGAATCGGAACCCTCAGACCCACAGCCGCAAGGCACTGGATTCTGGCAGC  
ACCCTGAAGGAGCTGGGAAGTAAGTTCTTCCCCAGCCTCCAGATAAGAGCCCCGCCGGCC  
AATCCCTTCATTTCAACCTAAAGAGACCCTAAGCAGAGAACCTAGCTGAGCCACTCCTGA  
CCTACAAAGTTGTGACTTAATAAATGTGTGCTTTAAGCTGCCAAAAAAAAAAAA

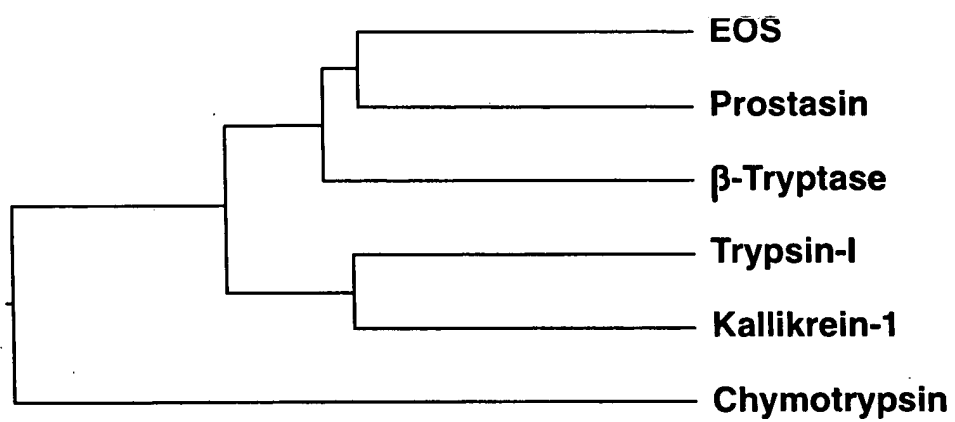
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**FIG. 1B****Amino Acid sequence of EOS protease (SEQ.ID.NO.:7)**

Met	Arg	Gly	Val	Ser	Cys	Leu	Gln	Val	Leu	Leu	Leu
Leu	Val	Leu	Gly	Ala	Ala	Gly	Thr	Gln	Gly	Arg	Lys
Ser	Ala	Ala	Cys	Gly	Gln	Pro	Arg	Met	Ser	Ser	Arg
Ile	Val	Gly	Gly	Arg	Asp	Gly	Arg	Asp	Gly	Glu	Trp
Pro	Trp	Gln	Ala	Ser	Ile	Gln	His	Pro	Gly	Ala	His
Val	Cys	Gly	Gly	Ser	Leu	Ile	Ala	Pro	Gln	Trp	Val
Leu	Thr	Ala	Ala	His	Cys	Phe	Pro	Arg	Arg	Ala	Leu
Pro	Ala	Glu	Tyr	Arg	Val	Arg	Leu	Gly	Ala	Leu	Arg
Leu	Gly	Ser	Thr	Ser	Pro	Arg	Thr	Leu	Ser	Val	Pro
Val	Arg	Arg	Val	Leu	Leu	Pro	Pro	Asp	Tyr	Ser	Glu
Asp	Gly	Ala	Arg	Gly	Asp	Leu	Ala	Leu	Leu	Gln	Leu
Arg	Arg	Pro	Val	Pro	Leu	Ser	Ala	Arg	Val	Gln	Pro
Val	Cys	Leu	Pro	Val	Pro	Gly	Ala	Arg	Pro	Pro	Pro
Gly	Thr	Pro	Cys	Arg	Val	Thr	Gly	Trp	Gly	Ser	Leu
Arg	Pro	Gly	Val	Pro	Leu	Pro	Glu	Trp	Arg	Pro	Leu
Gln	Gly	Val	Arg	Val	Pro	Leu	Leu	Asp	Ser	Arg	Thr
Cys	Asp	Gly	Leu	Tyr	His	Val	Gly	Ala	Asp	Val	Pro
Gln	Ala	Glu	Arg	Ile	Val	Leu	Pro	Gly	Ser	Leu	Cys
Ala	Gly	Tyr	Pro	Gln	Gly	His	Lys	Asp	Ala	Cys	Gln
Gly	Asp	Ser	Gly	Gly	Pro	Leu	Thr	Cys	Leu	Gln	Ser
Gly	Ser	Trp	Val	Leu	Val	Gly	Val	Val	Ser	Trp	Gly
Lys	Gly	Cys	Ala	Leu	Pro	Asn	Arg	Pro	Gly	Val	Tyr
Thr	Ser	Val	Ala	Thr	Tyr	Ser	Pro	Trp	Ile	Gln	Ala
Arg	Val	Thr	Ser	Asn	Ala	Ser	Arg				

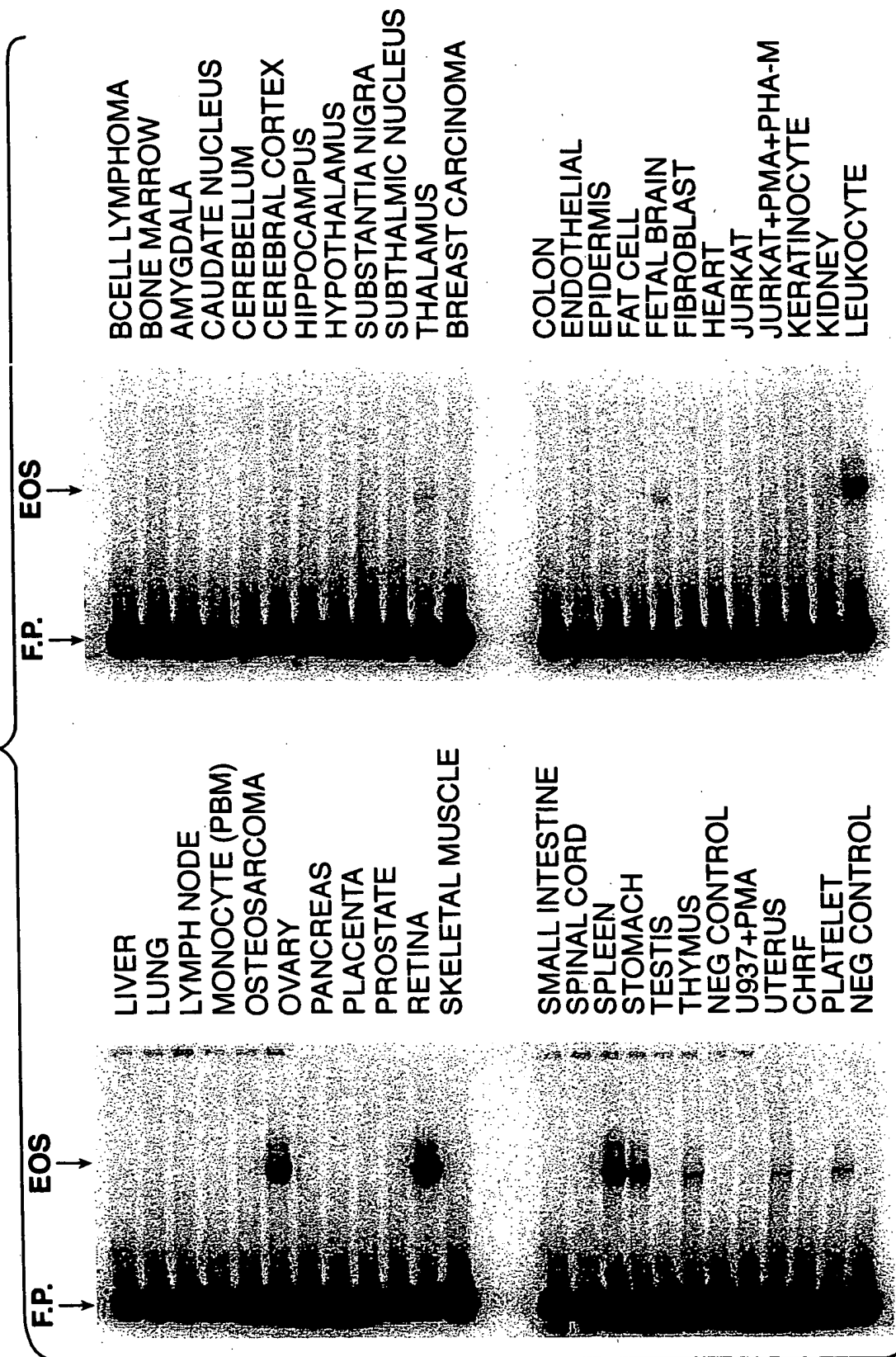
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**FIG. 2**



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FIG. 3



**FIG. 4A**Eco RI

GAATTCACCACCATGGACAGCAAAGGTTCTGTCGCAGAAATCCCGCCTGCT  
 1 -----+-----+-----+-----+-----+ 50  
 CTTAAGTGGTGGTACCTGTCGTTTCCAAGCAGCGTCTTTAGGGCGGACGA  
                   M D S K G S S Q K S R L L  
                   Prolactin Signal Sequence

CCTGCTGCTGGTGGTGTCAAATCTACTCTTGTGCCAGGGTGTGGTCTCCG  
 51 -----+-----+-----+-----+-----+ 100  
 GGACGACGACCACCACAGTTTAGATGAGAACACGGTCCCACACCAGAGGC  
                   L L L V V S N L L L C Q G V V S  
                   Prolactin Signal Sequence

Not I

ACTACAAGGACGACGACGACGTGGACGCGGCCGCTCTTGCTGCCCCCTTT  
 101 -----+-----+-----+-----+-----+ 150  
 TGATGTTCTGCTGCTGCTGCACCTGCGCCGGCGAGAACGACGGGGGAAA  
                   D Y K D D D D V D A A A L A A P F  
                   FLAG EK Pro

Xba I

GATGATGATGACAAGATCGTTGGGGGCTATGCTCTAGAGGACGGAGAGTG  
 151 -----+-----+-----+-----+-----+ 200  
 CTACTACTACTGTTCTAGCAACCCCGATACGAGATCTCCTGCCTCTCAC  
                   D D D D K I V G G Y A L E D G E W  
                   EK Pro

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**FIG. 4B**

201 GCGTGGCAGGCGAGCATCCAGCATCCTGGGGCACACGTGTGCGGGGGGT  
-----+-----+-----+-----+ 250  
CGGCACCGTCCGCTCGTAGGTCGTAGGACCCCGTGTGCACACGCCCCCA  
P W Q A S I Q H P G A H V C G G  
----- Protease EOS.CDS -----

251 CGCTCATCGCCCCCAGTGGGTGCTGACAGCGGCGCACTGCTTCCCCAGG  
-----+-----+-----+-----+ 300  
GCGAGTAGCGGGGGGTCACCCACGACTGTCGCCGCGTGACGAAGGGGTCC  
S L I A P Q W V L T A A H C F P R  
----- Protease EOS.CDS -----

301 AGGGCACTGCCAGCTGAGTACCGCGTGCGCCTGGGGGCGCTGCGTCTGGG  
-----+-----+-----+-----+ 350  
TCCCGTGACGGTCGACTCATGGCGCACGCGGACCCCGCGACGCAGACCC  
R A L P A E Y R V R L G A L R L G  
----- Protease EOS.CDS -----

351 CTCCACCTCGCCCCGCACGCTCTCGGTGCCCCTGCGACGGGTGCTGCTGC  
-----+-----+-----+-----+ 400  
GAGGTGGAGCGGGGCGTGCGAGAGCCACGGGCACGCTGCCACGACGACG  
S T S P R T L S V P V R R V L L  
----- Protease EOS.CDS -----

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**FIG. 4C**

401 C C C C G G A C T A C C G A G G A C G G G G C C C G C G G C G A C C T G G C A C T G C T G C A G  
-----+-----+-----+-----+-----+ 450  
G G G G C C T G A T G A G G C T C C T G C C C C G G G C G C C G C T G G A C C G T G A C G A C G T C  
P P D Y S E D G A R G D L A L L Q

Protease EOS.CDS

451 C T G C G T C G C C C G G T G C C C C T G A G C G C T C G C G T C C A A C C C G T C T G C C T G C C  
-----+-----+-----+-----+-----+ 500  
G A C G C A G C G G G C C A C G G G G A C T C G C G A G C G C A G G T T G G G C A G A C G G A C G G  
L R R P V P L S A R V Q P V C L P

Protease EOS.CDS

501 C G T G C C C G G C G C C C G C C C G C C C G G C A C A C C A T G C C G G G T C A C C G G C T  
-----+-----+-----+-----+-----+ 550  
G C A C G G G C C G C G G G C G G G C G G C G G G C C G T G T G G T A C G G C C C A G T G G C C G A  
V P G A R P P P G T P C R V T G

Protease EOS.CDS

551 G G G G C A G C C T C C G C C C A G G A G T G C C C C T C C C A G A G T G G C G A C C G C T A C A A  
-----+-----+-----+-----+-----+ 600  
C C C G T C G G A G G C G G G T C C T A C G G G G A G G G T C T C A C C G C T G G C G A T G T T  
W G S L R P G V P L P E W R P L Q

Protease EOS.CDS

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**FIG. 4D**

601 GGAGTAAGGGTGCCGCTGCTGGACTCGCGCACCTGCGACGGCCTCTACCA 650  
-----+-----+-----+-----+-----+  
CCTCATTCCCACGGCGACGACCTGAGCGCGTGGACGCTGCCGGAGATGGT  
G V R V P L L D S R T C D G L Y H

Protease EOS.CDS

651 CGTGGGCGCGGACGTGCCCCAGGCTGAGCGCATTGTGCTGCCTGGGAGTC 700  
-----+-----+-----+-----+-----+  
GCACCCGCGCCTGCACGGGGTCCGACTCGCGTAACACGACGGACCCTCAG  
V G A D V P Q A E R I V L P G S

Protease EOS.CDS

701 TGTGTGCCGGCTACCCCCAGGGCCACAAGGACGCCTGCCAGGGTGATTCT 750  
-----+-----+-----+-----+-----+  
ACACACGGCCGATGGGGGTCCCGGTGTTCTGCGGACGGTCCCCTAAGA  
L C A G Y P Q G H K D A C Q G D S

Protease EOS.CDS

751 GGGGGACCTCTGACCTGCCTGCAGTCTGGGAGCTGGGTCCTGGTGGGCGT 800  
-----+-----+-----+-----+-----+  
CCCCCTGGAGACTGGACGGACGTCAGACCCTCGACCCAGGACCACCCGCA  
G G P L T C L Q S G S W V L V G V

Protease EOS.CDS

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## FIG. 4E

801 GGTGAGCTGGGGCAAGGGTTGTGCCCTGCCCAACCGTCCAGGGGTCTACA  
-----+-----+-----+-----+ 850  
CCACTCGACCCCGTTCCCAACACGGGACGGGTGGCAGGTCCCCAGATGT  
V S W G K G C A L P N R P G V Y

Protease EOS.CDS

851 CCAGTGTGGCCACATATAGCCCCTGGATTCAGGCTCGCGTCACTTCTAAT  
-----+-----+-----+-----+ 900  
GGTCACACCGGTGTATATCGGGGACCTAAGTCCGAGCGCAGTGAAGATTA  
T S V A T Y S P W I Q A R V T S N

Protease EOS.CDS

Xba I  
901 GCTTCTAGATACCCCTACGATGTGCCCGATTACGCCGCTAGACATCACCA  
-----+-----+-----+-----+ 950  
CGAAGATCTATGGGGATGCTACACGGGCTAATGCGGCGATCTGTAGTGGT  
A S R Y P Y D V P D Y A A R H H H

HA/HIS-TAG

Not I  
951 TCACCATCACTAGCGGCCGCTTCCCTTTAGTGAGGGTTAATGCTTCGAGC  
-----+-----+-----+-----+ 1000  
AGTGGTAGTGATCGCCGGCGAAGGGAAATCACTCCCAATTACGAAGCTCG  
H H H \*

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**FIG. 4F**

1001 AGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGC  
-----+-----+-----+-----+ 1050  
TCTGTACTATTCTATGTAACCTACTCAAACCTGTTTGGTGTGATCTTACG

SV40 Late pA

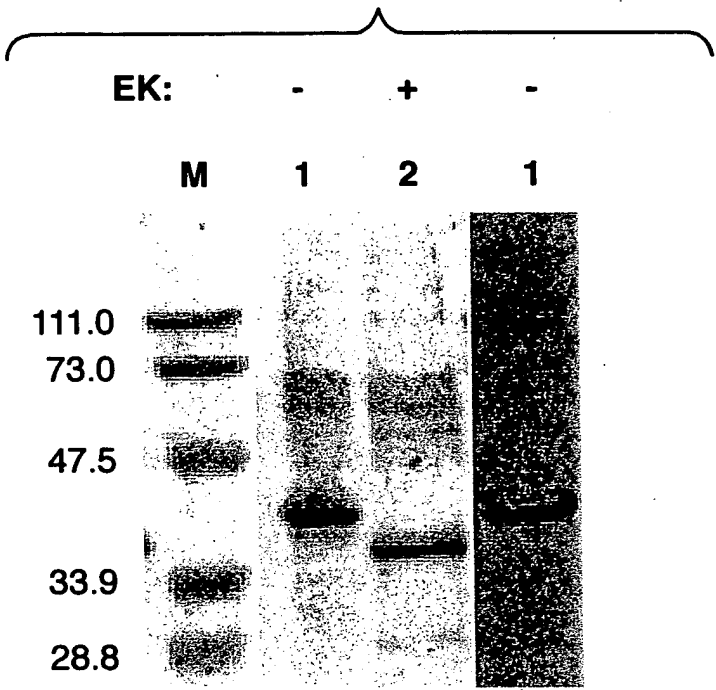
1051 AGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTT  
-----+-----+-----+-----+ 1100  
TCACTTTTTTTACGAAATAAACACTTTAAACACTACGATAACGAAATAAA

SV40 Late pA

1101 GTAACCATTATAAGCTGCAATAAACAAGTT  
-----+-----+ 1130  
CATTGGTAATATTTCGACGTTATTTGTTCAA

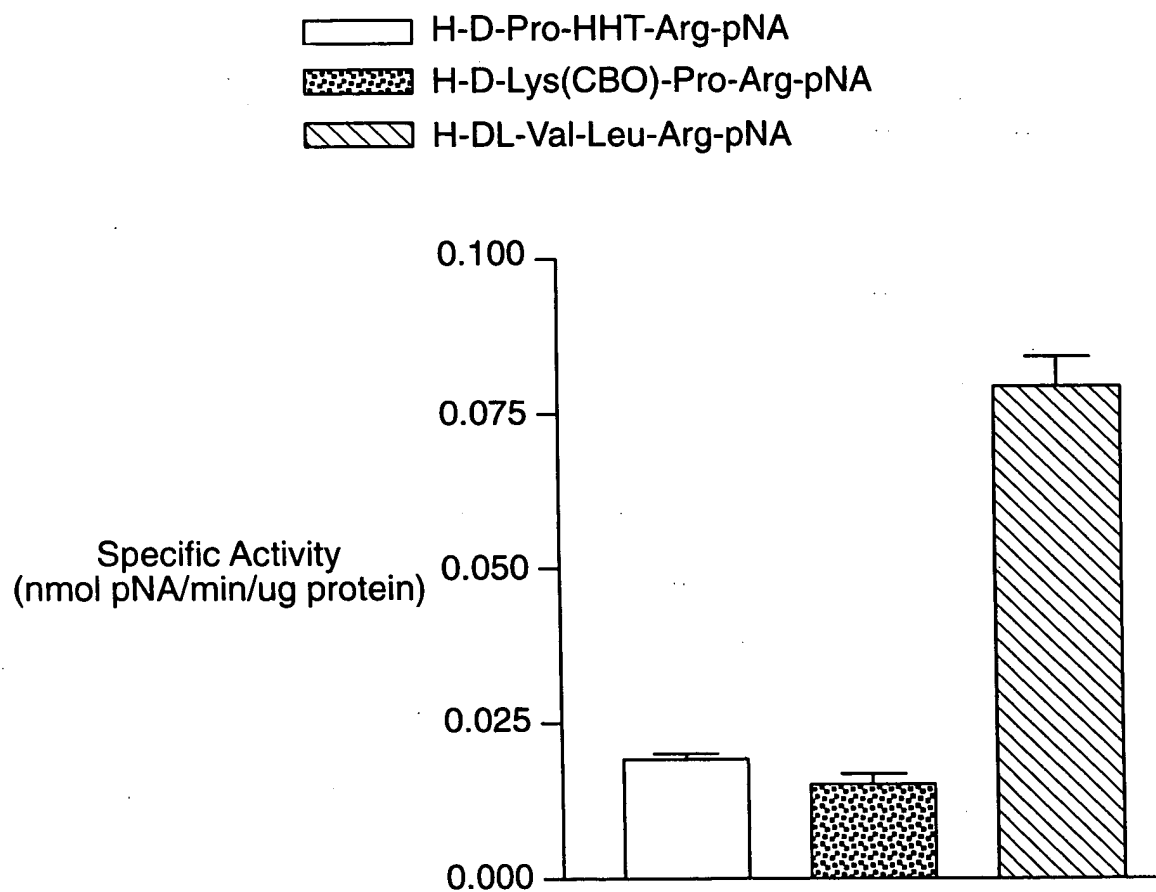
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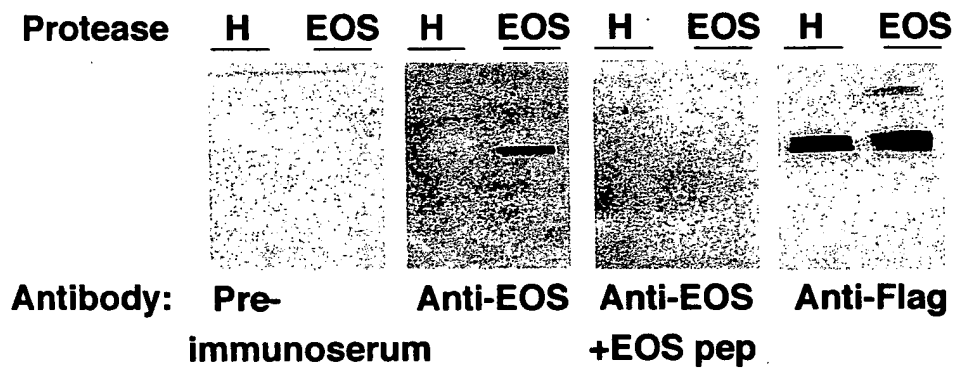
**FIG. 5**



**Protease: PFEK2-EOS-6XHIS**

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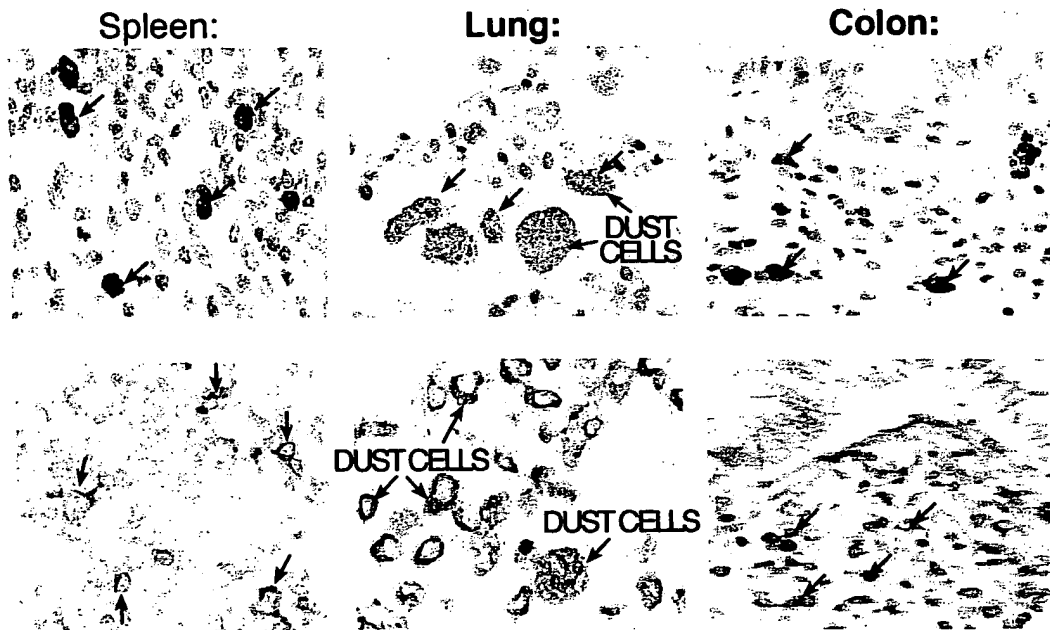
**FIG. 6****Protease Activity of EOS**

**FIG. 7****Anti-EOS Antiserum Immunoblot Characterization**

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# FIG. 8

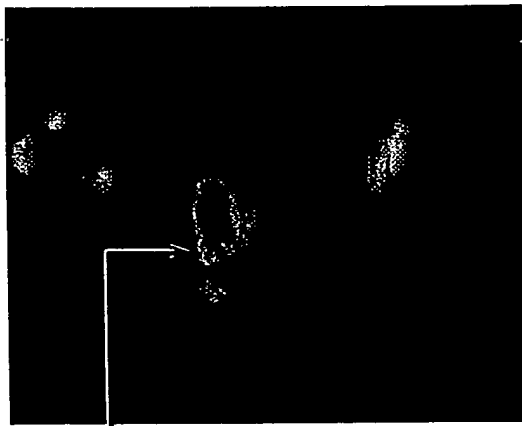
Localization of EOS protein (top) and mRNA (bottom)  
in human spleen, lung, and colon



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## FIG. 9

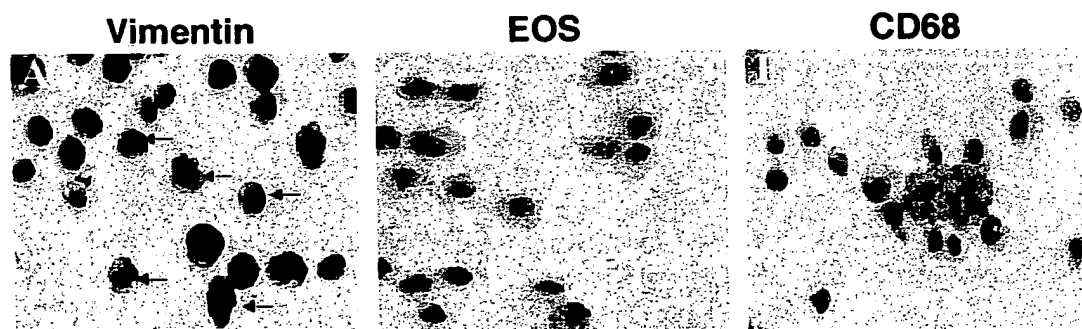
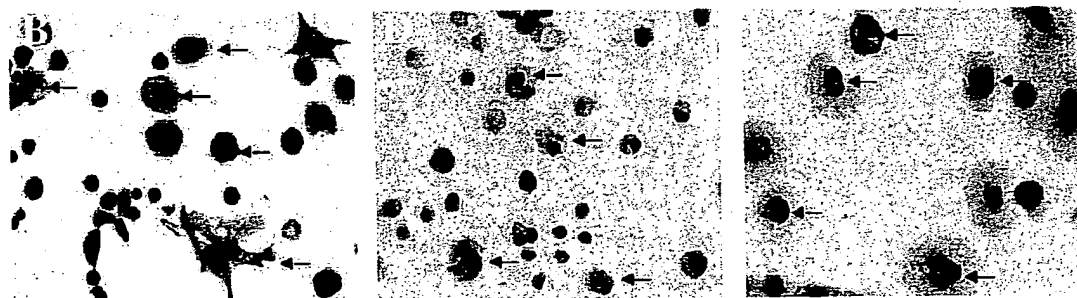
Double immunofluorescence (IF:IF) of  
EOS and macrophage marker:CD68



**EOS in human colon**



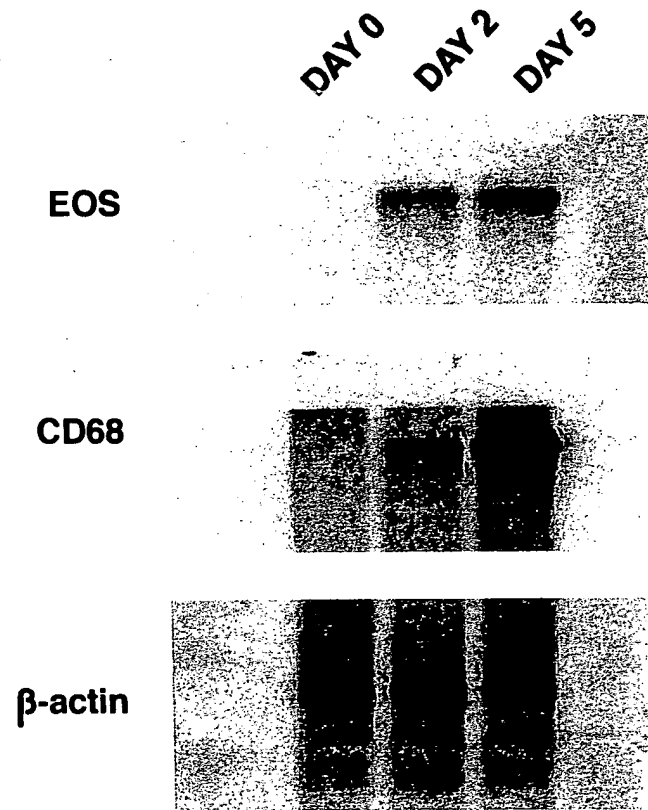
**CD68 in human colon**

**FIG. 10****Up-regulation of EOS protein by PMA in U937 cells****Untreated U937 cells:****PMA treated U937 cells:**



**FIG. 11**

Up-regulation of EOS mRNA by PMA in U937 cells



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